LACTOSE HYDROLYSIS IN MILK AND MILK PRODUCTS BY BOUND FUNGAL BETA-GALACTOSIDASE

J. H. WOYCHIK AND M. V. WONDOLOWSKI
Eastern Regional Research Laboratory[1]
Philadelphia, Pennsylvania 19118

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(Received for publication August 14, 1972)

ABSTRACT

The β-galactosidase of *Aspergillus niger* was immobilized by glutaraldehyde coupling to porous glass beads and the bound enzyme evaluated for its applicability to hydrolysis of lactose in milk and milk products. Lactose in sweet whey and skim milk was hydrolyzed at approximately one-third the rate in acid whey. Non-lactose solids inhibited β-galactosidase activity. Greater efficiency of lactose hydrolysis was obtained with the bound enzyme in column operations than in stirred batch reactors.

Use of β-galactosidases to hydrolyse lactose in milk and milk products is a subject of renewed interest *(1, 7, 8)*. Applications are readily apparent not only for the food industry, but also to prepare low-lactose dairy products intended for use by lactose intolerant individuals. Nonfat dry milk and whey powder products with portions of their lactose hydrolyzed have been prepared with good flavor, appearance, and stability *(7)*.

The purity, availability, and cost of β-galactosidases become important economic considerations in any large scale lactose-hydrolysis process. Although satisfactory hydrolysis is obtainable through addition of free enzymes, their one-time use appears uneconomical. Recent developments in enzyme immobilization *(3)* permit continuous extended use of the bound enzymes and can reduce costs significantly.

A comparative evaluation of microbial β-galactosidases indicates that the fungal enzyme appears more suited for use in immobilized systems than the yeast or bacterial enzymes. The *Aspergillus niger* β-galactosidase (β-D-galactoside galactohydrolase EC 3.2.1.23) offers several distinct advantages, namely, an operational half-life of perhaps several months, high heat stability, and freedom from metal requirements. Admittedly, use of the enzyme (pH optimum 4.0) appears to be limited primarily to acid whey products since skim milk (pH 6.8) and rennet wheys (pH 6.3) fall on the low end of its effective pH range. Further, the competitive inhibition exerted by the liberated galactose *(9)* markedly diminishes the efficiency of the enzyme during the continuous hydrolysis of lactose. Nevertheless, the applicability of immobilized *A. niger* β-galactosidase for the hydrolysis of lactose in several milk products will be evident from data in this report.

MATERIALS AND METHODS

β-Galactosidase

A partially purified preparation of the β-galactosidase of *A. niger* (Lactase LP) was a gift of Wallerstein Laboratories and was used without further purification.

Preparation of bound enzymes

The enzyme preparations were attached to Corning's Controlled Pore[2] glass beads (pore size 700 ± 70A, 120-200 mesh) by glutaraldehyde crosslinking according to the procedure reported by Robinson et al. *(5)*. The glass beads were immersed in a 2% solution of 3-aminopropyltriethoxysilane in acetone for 24 hr at 45 C. Aminoalkylsilane glass was stirred in a cold 1% aqueous solution of glutaraldehyde for 30 min, rinsed with water, and then suspended in cold phosphate buffer (pH 7.5) containing the β-galactosidase. After 2 hr the glass was rinsed with several volumes of H_2O, 0.5 M NaCl, and finally with 0.1 M acetate buffer, pH 4. No soluble β-galactosidase activity could be eluted from the glass following the washing procedures. Protein analysis of the supernatant and rinses by the Lowry procedure *(4)* indicated the coupling of 7 mg protein/g of glass. This preparation was used for the experiments reported below.

Enzyme activity and lactose hydrolysis

Enzymatic activity was determined by the amount of glucose released following incubation of either the free or bound β-galactosidases with substrate. Aliquots of digests containing the free enzyme were placed in a boiling water bath for 3 min to stop the activity before assay for glucose content. Activities of the immobilized enzymes were determined by pumping substrate through columns of bound enzyme or by incubation with a suspension maintained by overhead stirring. Appropriate aliquots were analyzed directly for glucose content by a glucose oxidase procedure *(2)* calibrated with standard glucose concentrations.

Skimmilk and whey samples

Raw skimmilk was obtained from a commercial dairy; the rennet and acid wheys were prepared by laboratory procedures. Products were pasteurized by holding at 65 C for 30 min.

RESULTS AND DISCUSSION

Although use of β-galactosidase preparations obtained by affinity chromatography *(6, 9)* would per-

[1]Agricultural Research Service, U. S. Department of Agriculture.

[2]Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. THE EFFECT OF FLOW-RATE ON THE HYDROLYSIS OF LACTOSE IN VARIOUS LACTOSE-CONTAINING SUBSTRATES[1]

Substrate[2]	Lactose hydrolyzed (%)	
	70 ml/hr	35 ml/hr
5% Lactose, pH 4.7	49	65
Acid whey, pH 4.7	43	61
Rennet whey, pH 6.6	15	24
Skim milk, pH 6.6	12	23

[1]A 4-cc column of bound enzyme operating at 37 C.
[2]Lactose content adjusted to 5% in all samples.

TABLE 2. INFLUENCE OF NON-LACTOSE ACID WHEY SOLIDS ON THE RATE OF LACTOSE HYDROLYSIS[1]

Non-lactose solids (%)	Lactose hydrolyzed[2] (%)
3.8	24.1
2.5	24.9
1.5	27.2
1.0	29.7

[1]A 4-cc column of bound enzyme operating at 100 ml/hr at 37 C.
[2]Lactose content adjusted to 10% in all samples.

TABLE 3. INFLUENCE OF SOLIDS CONTENT ON LACTOSE HYDROLYSIS IN ACID WHEY[1]

Total solids (%)	Lactose hydrolyzed (%)
5	22.2
10	17.8
20	12.6
30	9.1

[1]A 4-cc column of bound enzyme operating at 100 ml/hr at 37 C.

mit the binding of more enzymatic activity to the glass, it was believed important to evaluate an enzyme preparation as commercially available. The specific activity of the partially purified preparation was half that of the affinity purified *A. niger* β-galactosidase. The β-galactosidase was bound to the alkylamine glass using the glutaraldehyde procedure which is substantially simpler and more rapid than diazo coupling (9).

The bound enzyme had the same functional and stability properties as the free enzyme and retained approximately 75% of its original activity. The pH activity curve in Fig. 1a shows that the enzyme at pH 4.5 possesses 80% of its optimum activity, but only 10% at pH 6.5. Thus the relative efficiency of the enzyme for hydrolysis of lactose in milk products decreases significantly above pH 5.0. The effect of temperature on enzymatic activity is shown in Fig. 1b. The fact that maximum activity occurs at 55 C offers an advantage in that operations at that temperature would be essentially free of most microbial growth.

As was shown in another publication (9), galactose is a competitive inhibitor of the *A. niger* β-galactosidase. This inhibition increased during the course of lactose hydrolysis and substantially reduced the enzyme efficiency. For example, 0.01 M galactose causes a 60% inhibition in the hydrolysis of 0.1 M lactose. Thus, the hydrolysis rate is not proportional to the amount of enzyme added or to the time of exposure. This is readily apparent from data in Table 1 which show the effect of flow rate on hydrolysis

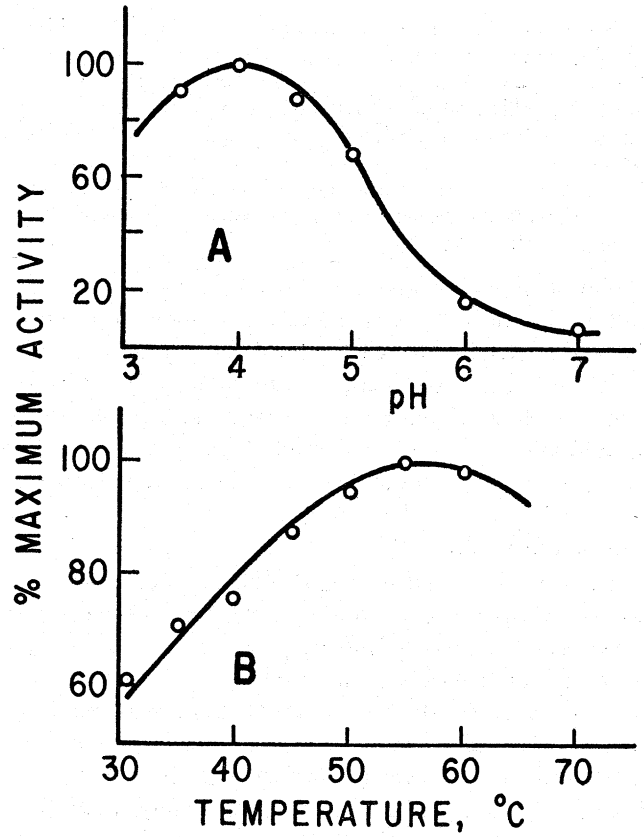

Figure 1. Influence of pH (A) and temperature (B) on the activity of *A. niger* β-galactosidase.

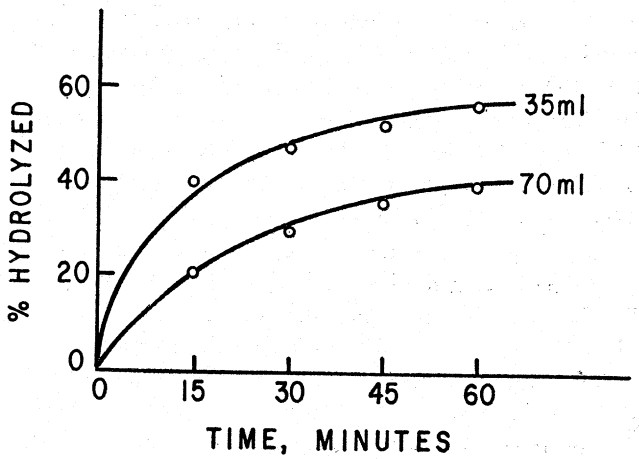

Figure 2. Influence of substrate volumes on the hydrolysis rates of 5% lactose (pH 4, 37 C) by stirred enzyme suspensions.

of various lactose containing substrates by a 4-cc column of immobilized enzyme. Doubling the enzyme exposure time of skim milk and sweet whey increased the hydrolysis rate 60-90%. However, because of the increased amounts of lactose hydrolyzed, and consequently the greater galactose inhibition, the increase did not exceed 42% in the acid products.

The extent of lactose hydrolysis in the substrates shown in Table 1 was dependent largely on their pH, with the neutral substrates being hydrolyzed at approximately one-third the rate of the acid substrates. It appeared that the non-lactose solids had an effect on the hydrolysis rates. This is most evident in the comparison between the values obtained for 5% lactose and acid whey. This effect was investigated further by determining lactose hydrolysis rates at various concentrations of non-lactose solids. Lyophilized acid whey was reconstituted to various concentrations and the lactose content of all samples adjusted to 10%. The effects on the hydrolysis rates were determined with a 4-cc column of immobilized enzyme. Data presented in Table 2 show that the hydrolysis rates increased as the solids content decreased. These results are similar to data obtained by Wendorf et al. *(7)* using solutions of *Saccharomyces fragilis* β-galactosidase. Data in Table 3 show that as the total solids increase in whey concentrates, increased amounts of lactose are hydrolyzed, although the percent of total lactose hydrolyzed decreases.

Immobilized enzymes can be used either batchwise or in column operations. However, with β-galactosidase, more efficient lactose hydrolysis was obtained by continuous-flow column operation compared to batch treatments with stirred suspensions of immobilized enzyme. The influence of substrate volumes on the hydrolysis rates of 5% lactose (pH 4, 37 C) by stirred enzyme suspensions is shown in Fig. 2. At the end of 1 hr, 56% lactose hydrolysis was obtained with 35 ml of substrate and 39% with 70 ml. In a column operation, the same 4-cc of bound enzyme hydrolyzed 73% of the lactose at 35 ml/hr and 53% at 70 ml/hr. These differences in amounts of lactose hydrolyzed can be attributed to the fact, that in a batch operation, all of the enzyme is exposed to increasing levels of galactose inhibition whereas, in a column operation, part of the enzyme is operating at higher substrate concentrations and lower levels of galactose inhibition.

A comparison of the lactose hydrolysis rates in raw and pasteurized acid wheys, skimmilks, and whole milks indicated that no inhibition of enzyme activity was operative in the raw samples compared with those pasteurized at 65 C for 30 min. This is in contrast to the reported inhibition of the β-galactosidase of *S. fragilis* by raw milk products. *(7)*.

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